NELSON LABORATORIES

Sponsor:
Rose Cattolico
University of Washington
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Seattle, WA 98195

# ISO/USP Medical Device Acute Systemic Toxicity Test in Mice

**Summary:** Enclosed is the final report for the testing we coordinated for you. The information is retained by the testing laboratory.

| | |
|---|---|
| Test Article: | DLE-14-48-3 |
| Nelson Laboratory Number: | 864523 |
| Testing Lab: | Sinclair Research |

**Results:** The test article (device) extracts did not cause acute adverse effects under the conditions of this assay.

If you have any questions, please feel free to call or email any of our Subcontracting personnel at 801-290-7500 or subcontracting@nelsonlabs.com. Thank you for testing with Nelson Laboratories, Inc.

Subcontracting Project Coordinator Jennifer Shaw, B.S.

27 Jan 2016
Date

sinclair research

A2LA ACCREDITED
Biological Testing Cert #3221.01

# FINAL REPORT

| | |
|---|---|
| **STUDY TITLE:** | **ISO/USP Medical Device Acute Systemic Toxicity Test in Mice** |
| **PROTOCOL NUMBER:** | D11006 (Version 5) |
| **STUDY NUMBER:** | D11006.046-208 |
| **TEST ARTICLE NAME:** | DLE-14-48-3 |
| **TEST ARTICLE LOT NO:** | N/A |
| **TEST FACILITY:** | Sinclair Research Center (SRC), LLC.<br>(AAALAC Accredited)<br>562 State Road DD<br>Auxvasse, MO 65231, USA<br>Phone: (573) 387-4400<br>Fax: (573) 387-4404 |
| **SPONSOR:** | Nelson Laboratories, Inc.<br>6280 South Redwood Road<br>Salt Lake City, UT 84123 |
| **NELSON REFERENCE NO:** | 864523 |
| **DATA REQUIREMENTS:** | GLP |
| **DATE SAMPLE RECEIVED:** | 29-Dec-2015 |
| **STUDY INITIATION DATE:** | 29-Dec-2015 |
| **STUDY COMPLETION DATE:** | 25-Jan-2016 |
| **RESULTS SUMMARY:** | The test article (device) extracts did not cause acute adverse effects under the conditions of this assay. |

## QUALITY ASSURANCE UNIT SUMMARY

The study has been performed under Good Laboratory Practice regulations (FDA, 21 CFR, Part 58 - Good Laboratory Practice for Non-clinical Laboratory Studies) and in accordance with standard operating procedures and study protocol. The quality assurance unit inspected this study on the dates listed below. The report accurately reflects the raw data.

| Phase Inspected | Date Inspected | Date Reported to Study Director & Management |
|---|---|---|
| Protocol (Version 5) | 02,03-Oct-2013 | 03-Oct-2013 |
| Day 3 Body Weights and Clinical Observations | 14-Jan-2016 | 14-Jan-2016 |
| Draft Report and Data | 25-Jan-2016 | 25-Jan-2016 |
| Final Report | 25-Jan-2016 | 25-Jan-2016 |

Quality Assurance Auditor: Ashley Abernathy Date 25Jan16

Ashley Abernathy

## GOOD LABORATORY PRACTICES STATEMENT

The SRC study referenced in this report was conducted in compliance with Good Laboratory Practice (GLP) Regulations set forth in Title 21 Part 58 of the Code of Federal Regulations of the United States of America. Other portions of this study that were not performed by or under the direction of SRC, including the characterization and stability testing of the test article (device), were the responsibility of the sponsor and are exempt from this GLP statement.

Study Director: Rachael Mullins Date 25Jan16

Rachael Mullins, B.S., Scientist I

## KEY STUDY PERSONNEL

| | |
|---|---|
| Rachael Mullins, B.S. | Study Director |
| Susan Schnapp, RVT, ALAT | Test Facility Management |
| Chris Hanks, DVM, M.S., DACLAM | Senior Staff Veterinarian |
| Catherine Selby, M.S. | Director of Operations |
| Karen Curtis | Quality Control Manager |

## OBJECTIVE

The objective of this study was to evaluate the systemic toxicity of leachable compounds from the test article (device). This test was intended for medical devices with contact duration categorized as repeat exposure. This study was performed to meet the biocompatibility testing requirements of ISO 10993 Part 11.

## TEST ARTICLE IDENTIFICATION

| | |
|---|---|
| Test Article Name: | DLE-14-48-3 |
| Lot/Batch #: | N/A |
| Nelson Reference No.: | 864523 |
| SRC Test Article ID #: | 8557 |
| Sterile/ Non-sterile: | Non-Sterile |
| Storage Conditions: | Room temperature |
| Intended Use/Application: | Unknown |
| Description: | yellow liquid |
| MSDS/CofA: | N/A |

## CONTROL ARTICLE IDENTIFICATION

| | |
|---|---|
| Control Article Name: | Buffer solution |
| Lot/Batch #: | N/A |
| SRC Test Article ID #: | 8558 |
| Sterile/ Non-sterile: | Non-Sterile |
| Storage Conditions: | Room temperature |
| Intended Use/Application: | Unknown |
| Description: | clear liquid |
| MSDS/CofA: | N/A |

## TEST ARTICLE CHARACTERIZATION

The sponsor was responsible for all test article characterization data as specified in the GLP regulations. The identity, strength, stability, purity, and chemical composition of the test article were solely the responsibility of the sponsor. It was the responsibility of the sponsor to ensure that the test article submitted for testing was representative of the final product that was subjected to materials characterization.

## EXPERIMENTAL DESIGN

A total of ten animals were used for this study with five animals in each group. Test and control articles were intravenously injected in a single dose. Dose volumes were based on the individual body weights of each animal. Body weights were obtained prior to dose administration (within 1 day) and daily for at least 3 days after administration. Clinical observations were performed at pre-dose, immediately following dosing, at 4 (± 1) hours after dose administration, and thereafter daily for a period of at least 3 days. Animals were euthanized at the termination of the study.

## JUSTIFICATION FOR SELECTION OF THE TEST SYSTEM

Mice are recognized as a standard model to predict systemic toxicity in humans, and are recommended by the FDA for toxicity studies. For this reason, mice were used in this study to evaluate the potential systemic toxicity of the test article.

## INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)

The protocol and any amendments or procedures involving the care or use of animals on this study were reviewed and approved by SRC IACUC prior to the initiation of such procedures. IACUC Protocol Approval Date: 06-Oct-2014

## IDENTIFICATION OF TEST SYSTEM

| | | | | | | |
|---|---|---|---|---|---|---|
| Species/Strain: | *Mus musculus*, Albino CD-1 Mice | | | | | |
| Source: | Envigo | | | | | |
| Age: | 3.4 weeks | | | | | |
| Weight Range: | 20.9 – 26.7 g | | | | | |
| Gender: | Male | | | | | |
| Identification: | Ear notch and cage card | | | | | |
| No. of Animals Used: | Total: | 10 | Control: | 5 | Test: | 5 |
| In-life Termination: | Study Day 3 | | | | | |

## HUSBANDRY

| | |
|---|---|
| Acclimation: | Acclimated at least 5 days prior to dose administration. |
| Housing: | Animals were randomly group-housed (2-3 per cage) upon receipt. |
| Environment: | Room temperature and lighting were monitored and controlled while humidity was monitored, but not controlled. All data was maintained as facility records. |
| Food/Water: | Animals were provided *ad libitum* food and water daily. Food and water analyses are maintained as facility records and there were no known contaminants expected to interfere with the test results. |

## ANIMAL SELECTION AND RANDOMIZATION

All animals were randomly placed into group housing upon receipt. Animals were selected for study based on a physical examination performed by trained personnel.

## ANIMAL PREPARATION

All animals were weighed once prior to dose administration.

## TEST ARTICLE PREPARATION

The test article was dosed neat, no dilution or extraction was performed.

## TEST AND CONTROL ARTICLE ADMINISTRATION

All study animals were dosed at a level of 50 mL/kg. The dose volume of the test article was determined by the individual body weights collected prior to dose. The control and test articles were administrated via IV injection. A single dose was performed for each test animal.

## GENERAL AND CLINICAL OBSERVATIONS AND BODY WEIGHTS

General observations were performed twice daily. Clinical observations were performed at pre-dose, immediately following dose and at ~4 hour after dose administration. Thereafter, animals were observed and weighed daily for the duration of the study. Observations were recorded for each animal according to **Table 1**.

**Table 1: Classification of General Condition**

| Observations | Description |
|---|---|
| No side effects observed (no symptoms). | Normal |
| Slight motor function decline, respiratory difficulty or signs of abdominal irritation. | Slight |
| Moderate abdominal irritation, respiratory difficulty, motor function decline, blepharoptosis, or diarrhea. | Moderate |
| Collapse, cyanosis, tumor or marked abdominal irritation, diarrhea, blepharoptosis, respiratory difficulties. | Marked |
| Mortality | Dead |

## TERMINATION

All animals were euthanized with $CO_2$ at termination.

## EVALUATION CRITERIA

Data from the test and control groups were compared and the following criteria were used to determine whether or not the test article passed the ISO-based toxicity test:

**Negative:**
If none of the animals treated with the test article were to show a significantly greater biological reaction than the animals treated with the control article, the test article would have passed the test.

**Positive:**
If two or more animals treated with the test article were to die or show gross signs of toxicity (e.g. convulsions, prostration), or if three or more animals were to lose more than 10% of their body weight for an ISO-based test or 2 grams of body weight for a USP-based test, the test article would have failed the test.

**Repeat Assay:**
If any animals treated with the test article were to show slight signs of biological reaction, and no more than one animal were to die or show gross signs of toxicity, a repeat test would have been conducted using an additional 10 animals for each group.

## ASSAY VALIDITY

The assay was considered valid based on the animals being within the correct weight ranges at dosing and the fact that all animals appeared healthy at the start of the study.

## METHOD FOR CONTROL OF BIAS

Although the study was not blinded, the use of a control article prevented experimental bias.

## ARCHIVAL

The original final report and an electronic copy (PDF) of the final report is provided to the Sponsor and a copy of the original report, original protocol (including amendments, deviations, Test Requisition Form and attachments, if applicable) and all original in-life study specific raw data as well as pertinent in-life facility data are archived at Sinclair Research Center LLC.

## COMPLIANCE

The procedures including care, housing and handling of animals were performed in compliance with the ISO 10993 Biological Evaluation of Medical Devices – Part 2: Animal Welfare Requirements. The study was conducted and reported in compliance with the ISO 10993 Biological Evaluation of Medical Devices – Part 11: Tests for Systemic Toxicity, U.S. Food and Drug Administration Good Laboratory Practice (GLP) regulations set forth in 21 CFR Part 58, and in accordance with the global protocol and the associated Test Requisition Form, facility standard operating procedures, and USDA Policy 12.

## RESULTS

### General and Clinical Observations

General observations were performed twice daily. Clinical observations were performed at pre-dose, immediately following dose and at ~4 hour after dose administration. Thereafter, animals were observed daily for the duration of the study. Dose administrations were successful. All study animals appeared normal during the study period. The data is presented in **Table 2.**

### Body Weight and Body Weight Change

All body weight data was collected within the targeted time periods. The individual data is summarized in **Table 3** & **4**.

## ANALYSIS AND CONCLUSION

Based on the clinical observations and body weight evaluations, the test article (device) extracts did not show a significantly greater biological reaction than the control article extracts. In conclusion, the test article extracts did not cause acute systemic toxicity under the conditions of this assay.

**Table 2: Clinical Observations**

| Animal ID | Observation | | | | |
|---|---|---|---|---|---|
| | Post-Dose | 4 hr | Day 1 | Day 2 | Day 3 |
| **NS Control Group** | | | | | |
| 1M1:268-0 | N | N | N | N | N |
| 1M2:268-1 | N | N | N | N | N |
| 1M3:268-2 | N | N | N | N | N |
| 1M4:269-0 | N | N | N | N | N |
| 1M5:269-1 | N | N | N | N | N |
| **NS Extract Group** | | | | | |
| 2M1:270-0 | N | N | N | N | N |
| 2M2:270-1 | N | N | N | N | N |
| 2M3:270-2 | N | N | N | N | N |
| 2M4:271-0 | N | N | N | N | N |
| 2M5:271-1 | N | N | N | N | N |

N= Normal

**Table 3: Body Weight**

| Animal ID | Body Weight (g) & Body Weight Change (%) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Predose | Day 1 | | Day 2 | | Day 3 | |
| | | BW | BWC | BW | BWC | BW | BWC |
| **NS Control Group** | | | | | | | |
| 1M1:268-0 | 22.7 | 23.7 | 4.4 | 24.4 | 7.5 | 25.3 | 11.5 |
| 1M2:268-1 | 23.1 | 26.6 | 15.2 | 28.6 | 23.8 | 29.1 | 26.0 |
| 1M3:268-2 | 20.9 | 22.7 | 8.6 | 24.2 | 15.8 | 25.4 | 21.5 |
| 1M4:269-0 | 22.1 | 25.3 | 14.5 | 27.1 | 22.6 | 29.0 | 31.2 |
| 1M5:269-1 | 23.5 | 25.5 | 8.5 | 25.8 | 9.8 | 26.7 | 13.6 |
| **NS Extract Group** | | | | | | | |
| 2M1:270-0 | 24.9 | 27.4 | 10.0 | 28.6 | 14.9 | 29.0 | 16.5 |
| 2M2:270-1 | 23.8 | 25.6 | 7.6 | 26.8 | 12.6 | 27.3 | 14.7 |
| 2M3:270-2 | 26.7 | 29.3 | 9.7 | 30.8 | 15.4 | 31.0 | 16.1 |
| 2M4:271-0 | 25.7 | 28.7 | 11.7 | 29.5 | 14.8 | 30.8 | 19.8 |
| 2M5:271-1 | 25.0 | 26.5 | 6.0 | 28.0 | 12.0 | 29.0 | 16.0 |

BW= Body Weight; BWC= Body Weight Change (comparing to Predose BW)

**Table 4: Summary of Observations and Body Weight Changes**

| Group | Number of Animals | Dead Animals | Mortality (%) | Mean Body Weight Change (%) | | |
|---|---|---|---|---|---|---|
| | | | | Day 1 | Day 2 | Day 3 |
| **NS Control** | 5 | 0 | 0.0 | 10.2 | 15.9 | 20.8 |
| **NS Extract** | 5 | 0 | 0.0 | 9.0 | 13.9 | 16.6 |

**REFERENCES**


| | |
|---|---|
| SRC SOP | Sinclair Research Center, LLC, Standard Operating Procedure Manual |
| ISO 10993-2:2006/(R)2010 | Biological Evaluation of Medical Devices Part 2: Animal Welfare Requirements |
| ISO 10993-11:2006/(R)2010 | Biological Evaluation of Medical Devices Part 11: Tests for Systemic Toxicity |
| ISO 10993-12:2012 | Biological Evaluation of Medical Devices Part 12: Sample Preparation and Reference Materials |
| FDA 21 CFR-Part 58 | Good Laboratory Practice For Nonclinical Laboratory Studies |
| USDA Policy 12 | Consideration of Alternatives to Painful/Distressful Procedures |
| USP 88 | Biological Reactivity Tests, IN VIVO |